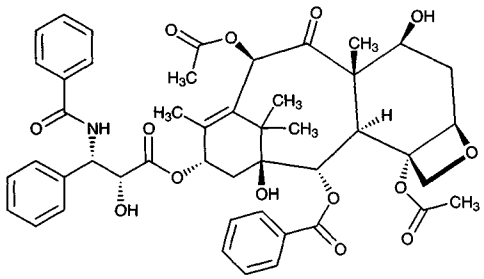
Paclitaxel

Molecular formula: $C_{47}H_{51}NO_{14}$
Molecular weight: 853.92
CAS Registry No.: 33069-62-4
Merck Index: 7117



SAMPLE

Matrix: blood
Sample preparation: Add 100 µL 10 µg/mL docetaxel in MeOH:water 50:50 and 5 mL MeCN: n-butyl chloride 20:80 to 1 mL plasma, vortex for 5 min, centrifuge at 4000 g for 5 min, evaporate the organic layer to dryness under a stream of nitrogen at 60°, reconstitute the residue with MeOH:water 50:50 with sonication for 1 min, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Inertsil ODS-80A (GL Science, Japan)
Mobile phase: MeOH:THF:water:ammonium hydroxide 60:2.5:37.5:0.1, pH adjusted to 6.0 with formic acid
Column temperature: 60
Flow rate: 1
Injection volume: 100
Detector: UV 230

CHROMATOGRAM

Retention time: 7.5
Internal standard: docetaxel (8.5)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, alizapride, codeine, dexamethasone, domperidone, lorazepam, metoclopramide, morphine, ranitidine
Interfering: paroxetine

KEY WORDS

pharmacokinetics; plasma

REFERENCE

Sparreboom,A.; de Bruijn,P.; Nooter,K.; Loos,W.J.; Stoter,G.; Verweij,J. Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1998**, *705*, 159–164.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 1 mL Baker Bond cyanopropyl SPE cartridge with 1 mL MeCN, 1 mL mobile phase, and 1 mL 50 mM KH_2PO_4. Free paclitaxel. Add 500 µL plasma or urine to the SPE cartridge, wash with 1 mL water, 1 mL MeCN:water 30:70, and 1 mL 50 mM KH_2PO_4, elute with two 300 µL portions of mobile phase, inject a 200 µL aliquot of the eluate. Total paclitaxel. To 500 µL plasma or urine add 500 µL MeOH:100 mM KH_2PO_4 50:50 (pH 7.5), hydrolyze at 22° for 20 h, stop the hydrolysis by adding an equal volume of 500 mM KH_2PO_4. Add the hydrolyzed sample to the SPE cartridge, wash with 1 mL water, 1 mL MeCN: water 30:70, and 1 mL 50 mM KH_2PO_4, elute with two 300 µL portions of mobile phase, inject a 200 µL aliquot of the eluate.

HPLC VARIABLES

Guard column: 4 × 4 4 µm LiChrospher 100 RP-8e
Column: 250 × 4 4 µm Superspher 60 RP-8e
Mobile phase: MeCN:175 mM pH 4.6 KH_2PO_4 buffer 55:45
Flow rate: 0.45

Injection volume: 200
Detector: UV 229

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
dog; plasma; SPE

REFERENCE
Fraier,D.; Cenacchi,V.; Frigerio,E. Determination of a new polymer-bound paclitaxel derivative (PNU 166945), free paclitaxel and 7-epipaclitaxel in dog plasma and urine by reversed-phase high-performance liquid chromatography with UV detection, *J.Chromatogr.A*, **1998**, *797*, 295–303.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Dilute sample with MeOH containing 0.1% acetic acid. Injections. Dilute sample with MeCN:water:acetic acid 70:30:0.1. Method 1. Column A, gradient A. Method 2. Column B, gradient B. Method 3. Column A, gradient C. Method 4. Column B, gradient D. Methods 1 and 2 are for bulk drug potency determination. Methods 3 and 4 are for the determination of the degradation profile for bulk and injections, potency and content uniformity of the injections and chromatographic purity profile of the bulk drug.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Curosil PFP (Phenomenex) (A), 250 × 4.6 5 μm Whatman TAC-1 (PFP) (Whatman) (B)
Mobile phase: Gradient A. MeCN:water from 40:60 by linear gradient at 0.444%/min until the paclitaxel peak elutes, return rapidly to initial composition and equilibrate. Gradient B. MeCN:water 38:62 for 12 min, by linear gradient at 4%/min until paclitaxel peak elutes, return to initial composition and equilibrate. Gradient C. MeCN:water from 40:60 to 60:40 over 45 min, to 80:20 over 5 min, hold for 5 min, return to initial composition and equilibrate. Gradient D. MeCN:water 38:62 for 12 min, to 70:30 over 8 min, hold for 15 min, return to initial composition and equilibrate.
Flow rate: 1 (column A), 1.5 (column B)
Injection volume: 15
Detector: UV 230

CHROMATOGRAM
Retention time: 24 (Method 1), 22.5 (Method 3), 17 (Method 4)
Limit of detection: 370 ng/mL (method 1), 310 ng/mL (method 2)
Limit of quantitation: 1.11 μg/mL (method 1), 930 ng/mL (method 2)

OTHER SUBSTANCES
Simultaneous: 13-acetyl-9-dihydrobaccatin, baccatin III, cephalomannine, 10-deacetyl baccatin III, N-debenzoyl-N-phenylacetyl taxol, 10-deacetyl-7-epi-taxol, 10-deacetyl taxol, 10-deacetyl-7-xylosyl taxol, 10-deacetyl-7-xylosyl taxol B, 10-deacetyl-7-xylosyl taxol C, 7-epi-taxol, taxinine M, taxol C, 7-xylosyl taxol
Noninterfering: degradation products, Cremophor EL

KEY WORDS
injections

REFERENCE
Shao,L.K.; Locke,D.C. Determination of paclitaxel and related taxanes in bulk drug and injectable dosage forms by reversed phase liquid chromatography, *Anal.Chem.*, **1997**, *69*, 2008–2016.

SAMPLE
Matrix: cell culture

Sample preparation: Pulverize dry callus cell culture, pass through 40-mesh sieve. Extract ultrasonically with MeOH:dichloromethane 10:1 for 30 min. Evaporate extract to dryness, dissolve residue in MeOH. Add an aliquot to a Sep-Pak C18 SPE cartridge, wash with water, wash with MeOH:water 30:70, elute with MeOH:water 85:15. Evaporate the eluate to dryness and redissolve in a minimum amount of MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18
Mobile phase: MeCN:MeOH:water 35:25:45
Flow rate: 1.0
Injection volume: 10
Detector: UV 227

CHROMATOGRAM
Retention time: 27
Limit of quantitation: 11.5 ng

OTHER SUBSTANCES
Extracted: baccatin, cephalomannine, 10-deacetyltaxol, 10-deacetylcephalomannine, baccatin, 10-deacetylbaccatin

KEY WORDS
SPE

REFERENCE
Wu,Y.; Zhu,W. High performance liquid chromatographic determination of taxol and related taxanes from Taxus callus cultures, *J.Liq.Chromatogr.*, **1997**, *20*, 3147–3154.

SAMPLE
Matrix: formulations
Sample preparation: Add paclitaxel injection to 0.9% NaCl injection or 5% dextrose injection to make a paclitaxel concentration of 0.3 or 1.2 mg/mL, mix thoroughly. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere RP18
Mobile phase: MeOH:water 90:10
Column temperature: 28
Flow rate: 1.3
Injection volume: 500
Detector: UV 273

CHROMATOGRAM
Retention time: 2.1

OTHER SUBSTANCES
Simultaneous: polyoxyethylated castor oil, diethylhexyl phthalate

KEY WORDS
injections

REFERENCE
Mazzo,D.J.; Nguyen-Huu,J.-J.; Pagniez,S.; Denis,P. Compatibility of docetaxel and paclitaxel in intravenous solutions with polyvinyl chloride infusion materials, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 566–569.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18

Mobile phase: MeCN:water 40:60
Flow rate: 2.25
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.95

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: plant
Sample preparation: Bark. Condition a Supelclean LC-18 SPE cartridge with MeOH and water. Homogenize (Ystral) 20 g dried bark with 100 mL MeOH. Sonicate the homogenate in a bath for 10 min, shake at 110 rpm for 30 min, filter through a glass filter. Re-extract residue with 50 mL MeOH, combine two MeOH extracts, evaporate to dryness at 40-45°, reconstitute the residue with 5 mL MeOH. Add a 500 μL aliquot to the SPE cartridge. Needles, clippings. Add 3 g sample to 100 mL chloroform:EtOH 50:50 (Caution! Chloroform is a carcinogen!), sonicate, filter though a glass filter, evaporate the filtrate to dryness, reconstitute the residue in 5 mL MeOH. Add a 500 μL aliquot to the SPE cartridge. Wash the cartridge twice with 2 mL portions of water, with 2 mL MeOH:water 20:80, and with 2 mL MeOH:water 50:50. Elute with 2 mL MeOH, evaporate the fractions to dryness in a speed vac, reconstitute the residue with two 100 μL aliquots of MeCN, inject 10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Lichrosorb RP-18
Column: 150 × 3.9 4 μm Novapak Phenyl
Mobile phase: Gradient. A was MeCN:50 mM ammonium acetate 30:70. B was MeCN:50 mM ammonium acetate 90:10. A:B 100:0, to 66:34 over 30 min, return to initial conditions over 2 min.
Flow rate: 0.8 (UV), 1 (MS)
Injection volume: 10
Detector: UV 227; MS, Finnigan MAT TSQ-70, electrospray, positive ion mode 150 V, sheath flow MeOH:water:acetic acid 80:20:1, mobile phase split 19:1 before MS

CHROMATOGRAM
Retention time: 22 (UV), 20 (MS)

OTHER SUBSTANCES
Extracted: baccatin III, cephalomannine, 10-DAB, 10-deacetyltaxol, 7-epi-10-deacetyltaxol, 7-epi-taxol, taxinine M, taxol C, 7-xylosyl-10-deacetyltaxol, 7-xylosyl-10-deacetyltaxol B, 7-xylosyl-10-deacetyltaxol C, 7-xylosyl-taxol

KEY WORDS
bark; needles; clippings; SPE

REFERENCE
Theodoridis,G.; Laskaris,G.; de Jong,C.F.; Hofte,A.J.P.; Verpoorte,R. Determination of paclitaxel and related diterpenoids in plant extracts by high-performance liquid chromatography with UV detection in high-performance liquid chromatography-mass spectrometry, *J.Chromatogr.A*, **1998**, *802*, 297–305.

SAMPLE
Matrix: plants
Sample preparation: Extract needles with MeOH, concentrate the extract under reduced pressure at <30°, partition concentrate with 0.8 volumes of water and 0.8 volumes of chloroform,

repeat the extraction with 0.6 volumes chloroform then 0.4 volumes chloroform, concentrate under reduced pressure, dry under vacuum at 35-40°, dissolve in MeCN/water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil C8
Mobile phase: MeCN:MeOH:water 50:10:40
Flow rate: 0.5
Detector: UV 254

KEY WORDS
needles; details of preparative HPLC in paper

REFERENCE
Rao,K.V.; Bhakuni,R.S.; Juchum,J.; Davies,R.M. A large scale process for paclitaxel and other taxanes from the needles of *Taxus* x *media Hicksii* and *Taxus floridana* using reverse phase column chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 427–447.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher diol
Mobile phase: Gradient. MeOH:carbon dioxide 8:92 for 3 min, to 28:72 over 25 min, to 35:65 over 5.7 min, maintain at 35:65 for 4 min.
Column temperature: 30
Flow rate: 2
Detector: UV 227

CHROMATOGRAM
Retention time: 15.77

OTHER SUBSTANCES
Simultaneous: impurities, degradation products

KEY WORDS
SFC; pressure 150 bar

REFERENCE
Jagota,N.K.; Nair,J.B.; Frazer,R.; Klee,M.; Wang,M.Z. Supercritical fluid chromatography of paclitaxel, *J.Chromatogr.A*, **1996**, *721*, 315–322.

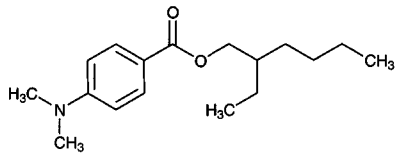
SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C-18 Guard Pak
Column: 100 × 8 10 μm Resolve C-18 radial compression (Waters)
Mobile phase: Gradient. MeCN:water from 45:55 to 100:0 over 20 min (exponential gradient), maintain at 100:0 for 5 min, re-equilibrate at initial conditions for 5 min.
Column temperature: 21
Flow rate: 2.5
Injection volume: 20
Detector: UV 227

CHROMATOGRAM
Retention time: 9.33

REFERENCE
Wenk,M.R.; Fahr,A.; Reszka,R.; Seelig,J. Paclitaxel partitioning into lipid bilayers, *J.Pharm.Sci.*, **1996**, *85*, 228–231.

Padimate O



Molecular formula: $C_{17}H_{27}NO_2$
Molecular weight: 277.41
CAS Registry No.: 21245-02-3
Merck Index: 3282

SAMPLE
Matrix: formulations
Sample preparation: Lotion. Weigh out lotion equivalent to about 90 mg oxybenzone, add 7 mL water, add MeOH slowly with vigorous shaking until total volume was 100 mL. Remove a 10 mL aliquot and make up to 100 mL with MeOH, filter (paper), discard first 5 mL of filtrate. Mix 4 mL filtrate and 1 mL 200 μg/mL sulfathiazole in MeOH, make up to 10 mL with MeOH, filter (0.45 μm), inject a 20 μL aliquot. Lipstick. Weigh out an amount equivalent to 25-90 mg oxybenzone, add 10 mL chloroform, add MeOH slowly with vigorous shaking until total volume was 100 mL. Remove a 10 mL aliquot and make up to 100 mL with MeOH, filter (paper), discard first 5 mL of filtrate. Mix 4 mL filtrate and 1 mL 200 μg/mL sulfathiazole in MeOH, make up to 10 mL with MeOH, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 25-37 μm Co:Pell ODS
Column: 250 × 4.6 10 μm Partisil PXS ODS-2
Mobile phase: MeCN:MeOH 10:90
Flow rate: 0.7
Injection volume: 20
Detector: UV 254

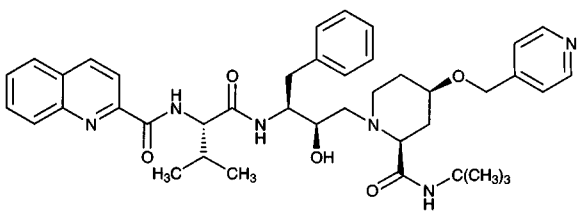
CHROMATOGRAM
Retention time: 7.4
Internal standard: sulfathiazole (3.9)
Limit of quantitation: 40 ng

OTHER SUBSTANCES
Simultaneous: oxybenzone, propyl paraben

KEY WORDS
lotion; lipstick; sun-screen

REFERENCE
Tan,H.S.I.; Sih,R.; Moseley,S.E.; Lichtin,J.L. Assay of mixtures of padimate-O and oxybenzone in sunscreen formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *291*, 275–282.

Palinavir



Molecular formula: $C_{41}H_{52}N_6O_5$
Molecular weight: 708.91
CAS Registry No.: 154612-39-2

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 1.5 M NaOH, extract three times with 2 mL portions of diethyl ether, vortex for 30 s, centrifuge at 3000 rpm for 10 min at 4°. Evaporate

the ether extract under a stream of nitrogen. Reconstitute the residue with 100 μL mobile phase. Inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 C-8 Nova-Pak
Mobile phase: MeCN:50 mM pH 3.0 potassium phosphate buffer 50:50 containing 0.1% dimethyloctylamine
Flow rate: 1.5
Injection volume: 80
Detector: UV 237

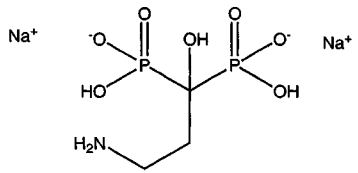
CHROMATOGRAM
Limit of detection: 2 nM

KEY WORDS
plasma; pharmacokinetics; rat

REFERENCE
Liard,F.; Jaramillo,J.; Paris,W.L.; Yoakim,C. Pharmacokinetic aspects of palinavir, an HIV protease inhibitor, in Sprague-Dawley rats, *J.Pharm.Sci.*, **1998**, *87*, 782–785.

Pamidronate

Molecular formula: $C_3H_9NNa_2O_7P_2$
Molecular weight: 279.01
CAS Registry No.: 57248-88-1, 109552-15-0 (pentahydrate), 40391-99-9 (free acid)
Merck Index: 7135



SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 500 mg Bakerbond quaternary amine SPE cartridge (J.T.Baker) with 2.5 mL water, 1 mL 1 mg/mL etidronate solution, 2.5 mL 100 mM nitric acid, and 5 mL water. Add 20 μL 5 mg/mL etidronate solution, 35 μL 100 mg/mL citric acid (to serum only), and 30 μL 2050 ng/mL neridronic acid solution to 1 mL serum or citrated plasma. Add 200 μL 20% trichloroacetic acid, vortex vigorously, centrifuge at 14000 g for 5 min. Add 30 μL 1 M calcium chloride, 40 μL 100 mM NaH_2PO_4, and 200 μL 1 M NaOH to the clear supernatant, vortex, centrifuge at 3900 g for 2 min, discard the liquid phase. Dissolve the residue in 25 μL 1 M HCl, dilute with 2.5 mL water, add to the SPE cartridge, wash with 1 mL water at 2 mL/min, wash with 4 mL water and 2.5 mL 10 mM nitric acid at 3 mL/min. Elute with 2.5 mL 100 mM nitric acid at 2.5 mL/min, evaporate the eluate to dryness under 0.6 bar nitrogen at 80° for 50-75 min, reconstitute the residue in 500 μL water, vortex. Add 50 μL 1 mg/mL etidronate, 75 μL triethylamine, and 500 μL 20 mg/mL 1-naphthylisothiocyanate in pyridine, mix by air bubbling to form a clear yellow solution. Heat the mixture at ca.80° for 15 min, extract twice with 2 mL 10 mg/mL tetrabutylammonium bromide in chloroform (Caution! Chloroform is a carcinogen!), mix the two phases by bubbling 3 mL air through the liquid, let the phases separate for 1 min, remove the lower organic layer. Mix 300 μL of the resulting sample and 90 μL 3% hydrogen peroxide, heat at ca.80° for 5 min, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 reversed-phase R2 (Chrompack)
Column: 100 × 4.6 3 μm Microspher C18 (Chrompack)
Mobile phase: MeCN:10 mM phosphate buffer containing 10 mM tetraoctylammonium bromide and 2 mM etidronate 62:38, pH 8.0
Flow rate: 0.8
Injection volume: 100
Detector: F ex 285 em 390

CHROMATOGRAM
Retention time: 12
Internal standard: neridronic acid (10.5)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

KEY WORDS
derivatization; plasma; serum; SPE

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Beijnen,J.H.; Vermeij,P. Semi-automatic liquid chromatographic analysis of pamidronate in serum and citrate plasma after derivatization with 1-naphthylisothiocyanate, *J.Chromatogr.B*, **1998**, *705*, 331–339.

SAMPLE
Matrix: formulations
Sample preparation: Add 10 μL 300 μg/mL IS to 100 μL 30 μL aqueous solution prepared from injections or tablets, vortex with 50 μL EtOH, 40 μL pyridine, 10 μL triethylamine, and 2 μL phenylisothiocyanate to yield a clear solution. Heat at 80° for 5 min and evaporate under nitrogen at 80°. Reconstitute the dry residue in 1 mL water, wash twice by vortexing with 1 mL 2 g/L tetrabutylammonium bromide in chloroform (Caution! Chloroform is an carcinogen!), centrifuge at 3900 g for 2 min, discard the organic layer. Add 90 μL 0.06% hydrogen peroxide to 900 μL of the aqueous phase, heat at 80° for 2 min, evaporate to dryness at 80°, reconstitute the residue in 100 μL mobile phase. Inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 R2 (Chrompack, Netherlands)
Column: 100 × 4.6 3 μm Microspher C18 (Chrompack, Netherlands)
Mobile phase: MeCN:buffer 22.5:77.5 (Buffer was 30 mM pH 7.0 phosphate buffer containing 5 mM tetrabutylammonium hydroxide and 2 mM etidronate.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 6.8
Internal standard: neridronic acid (9.0)
Limit of quantitation: 100 ng/mL

KEY WORDS
tablets; injections; derivatization

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Ramp-Koopmanschap,W.M.; Langebroek,R.H.; Vermeij,P. The determination of pamidronate in pharmaceutical preparations by ion-pair liquid chromatography after derivatization with phenylisothiocyanate, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 491–497.

SAMPLE
Matrix: solutions
Sample preparation: Vortex 100 μL 0.5 mg/mL pamidronate disodium in water, 15 μL triethylamine, and 100 μL 20 mg/mL naphthylisothiocyanate in pyridine to yield a clear solution. Heat in a sealed tube at 80° for 15 min. Wash twice with 1 mL 10 mg/ml tetrabutyl ammonium bromide in chloroform, discard the organic solvent. Add 10 μL 0.2% hydrogen peroxide solution and heat at 80°. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.0 5μm Chromspher C18 (Chrompack)
Mobile phase: MeCN:buffer 60:40 (Buffer was 20 mM phosphate buffer containing 5 mM tetraoctylammonium bromide + 500 μM etidronate (adsorption supressor).)
Column temperature: 30

Flow rate: 0.4
Injection volume: 20
Detector: UV 285; F ex 285 em 390

CHROMATOGRAM
Retention time: 11.5
Limit of detection: 5 ng/mL

KEY WORDS
derivatization

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Beijnen,J.H.; Vermeij,P. Derivatization of pamidronate and other amino(bis)phosphonates with different isothiocyanates prior to ion-pair liquid chromatography, *J.Chromatogr.A*, **1997**, *782*, 211–217.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Plasma or urine + 100 μL 25 μg/mL IS in 100 mM NaOH, adjust pH to 3 with concentrated HCl, filter (0.45 μm fluoro-membrane, Skan Model Acro LC 13). Add 0.5 mL 1.5 M trichloroacetic acid to the filtrate, centrifuge at 1500 g for 15 min. Remove the supernatant, add 20 μL 2.5 M calcium chloride solution, add 40 μL 500 mM NaH_2PO_4, adjust pH to 12.0 with 6.25 M NaOH then with 0.5 M NaOH, centrifuge at 1500 g for 15 min. Wash the precipitate with water, dissolve the precipitate in 200 μL 130 mM disodium EDTA adjusted to pH 10 with 6.25 M NaOH, add 100 μL 3 mg/mL fluorescamine in MeCN while vortexing vigorously, add 200 μL dichloromethane, extract, centrifuge at 1000 g for 3 min, remove the aqueous phase, inject a 10 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:1 mM disodium EDTA adjusted to pH 6.5 with 1 M NaOH 3:97
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 395 em 480

CHROMATOGRAM
Retention time: 3.9
Internal standard: 6-amino-1-hydroxypentilidenebisphosphonate (CGP 33 637) (4.8)
Limit of detection: 10 nM (plasma, urine)
Limit of quantitation: 800 nM (plasma), 700 nM (urine)

KEY WORDS
derivatization; pharmacokinetics; plasma

REFERENCE
Flesch,G.; Tominaga,N.; Degen,P. Improved determination of the bisphosphonate pamidronate disodium in plasma and urine by pre-column derivatization with fluorescamine, high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.*, **1991**, *568*, 261–266.

SAMPLE
Matrix: bone
Sample preparation: Dissolve 25 mg ground (<20 μm) bone in 2 mL 200 mM HCl, add 5 μg IS, vortex, let stand overnight at room temperature, centrifuge. Remove a 500 μL aliquot and add it to 1 mL 10 mM NaOH and 50 μL 1 M NaOH, centrifuge at 1000 g for 10 min, wash the

pellet with 1 mL water, centrifuge, discard the supernatant. Dissolve the pellet in 200 μL 200 mM phosphoric acid, add 250 μL 200 mM pH 10.3 EDTA in 200 mM NaOH, add 200 μL resin, vortex, centrifuge, filter (0.2 μm) a 550 μL aliquot of the supernatant, add 10 μL 10 M NaOH to the filtrate. Remove a 50 μL aliquot and add it to 50 μL 1 M pH 10.7 carbonate buffer, add 10 μL 1 mg/mL 2,3-naphthalenedicarboxaldehyde, add 10 μL 1 mg/mL N-acetyl-D-penicillamine, mix, let stand for 2 min, inject a 20-50 μL aliquot. (The resin was AG 50W-X8 (K^+ form) resin. Prepare the resin by adding 3 volumes of 1 M KOH to 200-400 mesh AG 50W-X8 cation-exchange resin H^+ form (Bio-Rad), stir for 30 s, decant the supernatant, repeat the procedure twice, wash five times with 3 volumes of water, store at 4°, before use wash 2 or 3 times with three volumes of water (J. Chromatogr. 1992, 584, 213).)

HPLC VARIABLES

Guard column: present but not specified
Column: 150 × 4.6 PLRP-S (Phenomenex)
Mobile phase: MeCN:25 mM pH 6.5 citrate-phosphate buffer 16:94
Flow rate: 1
Injection volume: 20-50
Detector: F ex 436 em 508

CHROMATOGRAM

Retention time: 5.0
Internal standard: 1-hydroxypentanylidene-1,1-bisphosphonate (Ciba-Geigy) CGP 38146 (13.5)
Limit of quantitation: 7.5 μg/g

KEY WORDS

derivatization

REFERENCE

King,L.E.; Vieth,R. Extraction and measurement of pamidronate from bone samples using automated pre-column derivatization, high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.B*, **1996**, *678*, 325–330.

SAMPLE

Matrix: formulations
Sample preparation: Dilute injections 100-fold, inject a 20 μL aliquot. Disintegrate a 5 mg tablet in 100 mL water, sonicate for 5 min, centrifuge an aliquot at 3600 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 10 μm IC-PAK Anion HC (Waters)
Mobile phase: 1.5 mM Nitric acid containing 0.5 mM copper(II) nitrate (Prepare column by pumping ILC Regenerant A (Waters) and 100 mM nitric acid for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM

Retention time: 2
Limit of detection: 400 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: alendronate, clodronate, etidronate, neridronate, olpadronate

KEY WORDS

derivatization; complexation; injections; tablets

REFERENCE

Sparidans,R.W.; Den Hartigh,J.; Vermeij,P. High-performance ion-exchange chromatography with in-line complexation of bisphosphonates and their quality control in pharmaceutical preparations, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1545–1550.

SAMPLE

Matrix: urine

Sample preparation: Filter dog urine through filter paper. 2 mL Urine + 100 μL IS in water, adjust pH to 3 with concentrated HCl, filter (0.45 μm fluoro-membrane, Skan Model Acro LC 13). Add 0.5 mL 1.5 M trichloroacetic acid to the filtrate, centrifuge at 1500 g for 15 min. Remove the supernatant, add 20 μL 2.5 M calcium chloride solution, add 40 μL 500 mM NaH_2PO_4, adjust pH to 12.0 with 6.25 M NaOH then with 0.5 M NaOH, centrifuge at 1500 g for 15 min. Wash the precipitate with water, dissolve the precipitate in 200 μL 130 mM disodium EDTA adjusted to pH 9 with 6.25 M NaOH, add 100 μL 1 mg/mL fluorescamine in MeCN while vortexing vigorously, add 200 μL dichloromethane, extract, centrifuge at 1000 g for 3 min, remove the aqueous phase, inject a 10 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:1 mM disodium EDTA adjusted to pH 6.5 with 1 M NaOH 3:97
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 395 em 480

CHROMATOGRAM

Retention time: 4
Internal standard: 6-amino-1-hydroxyhexylidenebisphosphonate (CGP 38 146) (8)
Limit of detection: 50 nM
Limit of quantitation: 1000 nM

KEY WORDS

derivatization; human; dog; pharmacokinetics

REFERENCE

Flesch,G.; Hauffe,S.A. Determination of the bisphosphonate pamidronate disodium in urine by pre-column derivatization with fluorescamine, high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.*, **1989**, *489*, 446–451.

SAMPLE

Matrix: urine

Sample preparation: Condition a 3 mL 500 mg Bakerbond quaternary amine SPE cartridge with 2.5 mL water, 1 mL 1 mg/mL etidronate, 2.5 mL 100 mM nitric acid, and two 2.5 mL portions of water. 2.5 mL Urine + 100 μL 1 mg/mL etidronate + 100 μL 2.16 μg/mL IS, mix, add 30 μL 1 M calcium chloride, vortex, add 25 μL portions of 1 M NaOH (vortexing after each addition) until a precipitate is clearly present, add 25 μL 1 M NaOH, vortex, centrifuge at 3900 g for 2 min. Remove the pellet and dissolve it in 50 μL 1 M HCl, add 2.5 mL water, add 50 μL 1 M NaOH, centrifuge. Remove the pellet and dissolve it in the minimum amount of 1 M HCl (30-50 μL), add 2.5 mL water, add 30 μL 1 M NaOH, centrifuge. Remove the pellet and dissolve it in the minimum amount of 1 M HCl (30-50 μL), add 2.5 mL water, add to the SPE cartridge, wash with two 2.5 mL portions of water, wash with 2.5 mL 10 mM nitric acid, elute with 2.5 mL 100 mM nitric acid. Evaporate the eluate to dryness under 0.8 bar nitrogen at 60° for 1.65-2 h, add 500 μL water, vortex. Remove a 250 μL aliquot and add it to 25 μL 1 mg/mL etidronate, add 40 μL triethylamine, add 250 μL 20 mg/mL 1-naphthylisothiocyanate in pyridine, vortex, heat at 80° for 15 min, cool, add 2 mL 10 mg/mL tetrabutylammonium bromide (?) in chloroform, vortex, centrifuge, discard the lower organic phase, repeat the procedure. Remove a 250 μL aliquot of the aqueous phase and add it to 75 μL 3% hydrogen peroxide (to convert the thiourea derivative to the corresponding urea), mix, heat at 80° for 5 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 R2 (Chrompack)
Column: 100 × 4.6 3 μm Microspher C18 (Chrompack)
Mobile phase: MeCN:buffer 65:35 (Buffer was 10 mM phosphate containing 10 mM tetraoctylammonium bromide and 2 mM etidronate (monosodium (1-hydroxyethylidene)bisphosphonate) (as adsorption suppressor), pH 7.6-7.9.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 100
Detector: F ex 285 em 390

CHROMATOGRAM
Retention time: 10
Internal standard: (3-amino-3-phenyl-1-hydroxypropylidene)bisphosphonic acid (17)
Limit of detection: 1 ng/mL
Limit of quantitation: 3 ng/mL

KEY WORDS
derivatization; SPE

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Beijnen,J.H.; Vermeij,P. Determination of pamidronate in urine by ion-pair liquid chromatography after derivatization with 1-naphthylisothiocyanate, *J.Chromatogr.B*, **1997**, *696*, 137–144.

Pancreatin

CAS Registry No.: 8049-47-6
Merck Index: 7137

SAMPLE
Matrix: solutions

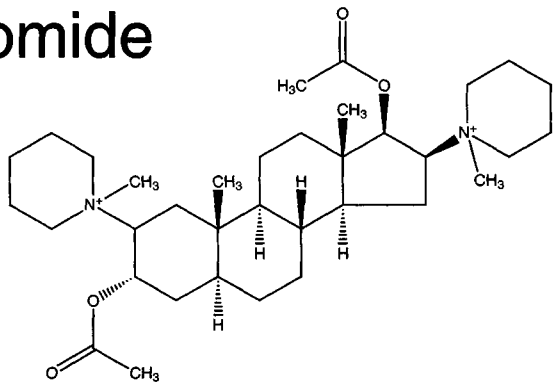
HPLC VARIABLES
Column: 250 × 4.6 Rexchrom 5 μm 300Å C8 (Regis)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B 100:0 to 0:100 over 40 min.
Flow rate: 1.5
Injection volume: 500
Detector: UV 214

CHROMATOGRAM
Retention time: 18-27 (multiple peaks)

REFERENCE
Baxter Scientific Products Catalog, 1990-1, p. 154.

Pancuronium bromide

Molecular formula: $C_{35}H_{60}Br_2N_2O_4$
Molecular weight: 732.68
CAS Registry No.: 15500-66-0
Merck Index: 7139
Lednicer No.: 2 163



SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 250 μL picric acid (1:10 dilution of saturated picric acid solution) + 250 μL vecuronium solution + 250 μL water + 5 mL dichloromethane:isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 μL MeCN: water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 μPorasil
Mobile phase: MeCN:2 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100
Detector: conductivity 2500 nS full scale

CHROMATOGRAM

Retention time: 5.7
Internal standard: vecuronium (4.7)
Limit of detection: 20 ng/mL

KEY WORDS

plasma

REFERENCE

Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.027

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 0.5% solution in the mobile phase, inject a 20 μL aliquot.

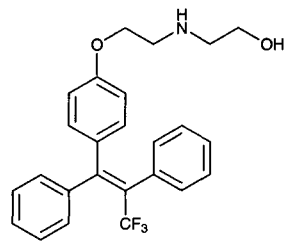
HPLC VARIABLES
Column: 250 × 4 5 μm SI 100 (Bio Separation Technologies)
Mobile phase: MeCN:100 mM sodium perchlorate 96:4
Flow rate: 1
Injection volume: 20
Detector: UV 213

CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Simultaneous: pipecuronium
Interfering: vecuronium

REFERENCE
Gazdag,M.; Babják,M.; Kemenes-Bakos,P.; Görög,S. Analysis of steroids. XLI. Ion-pair high-performance liquid chromatographic separation of quaternary ammonium steroids on silica, *J.Chromatogr.*, **1991**, *550*, 639–644.

Panomifene



Molecular formula: $C_{25}H_{24}F_3NO_2$
Molecular weight: 427.47
CAS Registry No.: 77599-17-8

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL phenyl SPE cartridge with five 1 mL portions of MeCN, 1 mL water, and 1 mL 2.5 mL/L triethylamine in 50 mM pH 3.0 phosphate buffer. 980 μL Plasma + 20 μL MeOH:water 50:50 + 10 μL 2 μg/mL tamoxifen in MeOH:water 50:50 + 1 mL MeCN, vortex for 1 min, centrifuge at -10° at 2500 g for 1 h. Remove a 1.6 mL aliquot of the supernatant and add it to 400 μL 2% heptanesulfonic acid in 50 mM pH 3.0 KH_2PO_4/phosphoric acid buffer, add to the SPE cartridge, wash with two 100 μL portions of MeCN:buffer 80:20,

wash with 50 μL 25 mM sulfuric acid, elute with five 100 μL portions of MeCN:buffer 80:20. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL MeCN:buffer 70:30, inject a 10-30 μL aliquot. (Buffer was 5 mM heptanesulfonic acid in 50 mM pH 3.0 phosphate buffer.)

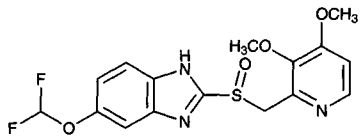
HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Si-100-S Phenyl (BST, Budapest)
Column: 250 × 4.6 10 μm Si-100-S Phenyl (BST, Budapest)
Mobile phase: MeCN:buffer 75:25 (Buffer was 50 mM pH 3.0 KH_2PO_4/phosphoric acid buffer containing 5 mM heptanesulfonic acid and 300 μL/L triethylamine. Temperature of MeCN was 60° and temperature of buffer was 80°.)
Flow rate: 1.2
Injection volume: 10-30
Detector: F ex 257 em 378 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID knitted PTFE coil irradiated by a mercury lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 5.73
Internal standard: tamoxifen (7.03)
Limit of detection: 1 ng/mL

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma; pharmacokinetics; SPE

REFERENCE
Erdélyi-Tóth,V.; Pap,E.; Kralovánszky,J.; Bojti,E.; Klebovich,I. Determination of panomifene in human plasma by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *668*, 419–425.

Pantoprazole



Molecular formula: $C_{16}H_{15}F_2N_3O_4S$
Molecular weight: 383.38
CAS Registry No.: 102625-70-7
Merck Index: 7146
Lednicer No.: 5 115

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 2000 g for 10 min, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 2 min backflush the contents of column A on to column B and start the gradient, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 25-40 μm LiChroprep RP-2; B 12 × 4.6 5 μm Hypersil RP-18 + 125 × 4.6 5 μm Hypersil RP-18
Mobile phase: A MeCN:50 mM pH 5 sodium acetate (Merck Extra Pure) buffer 10:90; B Gradient. MeOH:10 mM pH 6.5 ammonium phosphate buffer from 43:57 to 83:17 over 2 min, after 17 min flush at 100:0 for 2 min, re-equilibrate at initial conditions for 7 min.
Flow rate: A 1.5; B 1
Injection volume: 100
Detector: UV 290

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; column-switching

REFERENCE
Doyle,E.; McDowall,R.D.; Murkitt,G.S.; Picot,V.S.; Rogers,S.J. Two systems for the automated analysis of drugs in biological fluids using high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 67–77.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge serum or plasma at 2000 g for 10 min, inject a 200 μL aliquot on to column A and elute to waste with mobile phase A, after 2 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. Between runs flush column A with mobile phase A.

HPLC VARIABLES
Column: A 25-40 μm LiChroprep RP-2; B 12 × 4.6 5 μm Hypersil RP-18 + 125 × 4.6 5 μm Hypersil RP-18
Mobile phase: A 100 mM pH 5 sodium acetate buffer; B Gradient. MeOH:10 mM pH 6.5 $(NH_4)_2HPO_4$ 43:57 for 2 min, to 83:17 over 17 min, to 100:0 over 2 min, re-equilibrate for 7 min.
Flow rate: A 1.5; B 1
Injection volume: 200
Detector: UV 290

CHROMATOGRAM
Retention time: 16.5
Limit of detection: 4 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; plasma; dog; pharmacokinetics; column-switching

REFERENCE
Huber,R.; Muller,W.; Banks,M.C.; Rogers,S.J.; Norwood,P.C.; Doyle,E. High-performance liquid chromatographic determination of the H+/K+ ATPase inhibitor (BY 1023/SK&F 96,022) and its sulphone metabolite in serum or plasma by direct injection and fully automated pre-column sample clean-up, *J.Chromatogr.*, **1990**, *529*, 389–401.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in EtOH, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralpak AD
Mobile phase: Hexane:EtOH 80:20
Column temperature: 35
Flow rate: 1
Injection volume: 10-20
Detector: UV 302

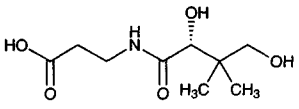
CHROMATOGRAM
Retention time: k' 6.10 (of first enantiomer)

OTHER SUBSTANCES
Simultaneous: lansoprazole, omeprazole, timoprazole

KEY WORDS
chiral; $\alpha = 1.19$

REFERENCE
Balmér,K.; Persson,B.-A.; Lagerström,P.-O. Stereoselective effects in the separation of enantiomers of omeprazole and other substituted benzimidazoles on different chiral stationary phases, *J.Chromatogr.A*, **1994**, *660*, 269–273.

Pantothenic acid



Molecular formula: $C_9H_{17}NO_5$
Molecular weight: 219.24
CAS Registry No.: 79-83-4 (D), 137-08-6 (Ca salt D), 6381-63-1 (Ca salt racemic), 599-54-2 (racemic)
Merck Index: 7147

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.772

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: food
Sample preparation: Completely dissolve 20 g of an elemental diet (Elental, Ajinomoto Co., Kawasaki) in 60 mL water at 50°, add 10 g sodium chloride, let stand at room temperature for 30 min. Dilute to 100 mL with water, wash with 10 mL hexane for 3 min to remove any oils. Inject a 20 µL aliquot of the aqueous layer onto column A and elute to waste with mobile phase A, after 4 min elute the contents of column A onto column B with mobile phase B, when the compounds of interest have moved onto column B remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 150 × 4.6 5 μm Capcellpak C18 (Shiseido, Tokyo); B 250 × 4.6 5 μm Capcellpak C18 (Shiseido, Tokyo)
Mobile phase: A MeCN:buffer 5:95; B MeCN:buffer 9:91 containing 1.5 mM sodium 1-heptanesulfonate (Buffer was water adjusted to pH 2.1 with phosphoric acid.)
Column temperature: 35
Flow rate: 1.2
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Limit of detection: ca. 5 ng

KEY WORDS
elemental diet; column switching

REFERENCE
Iwase,H. Determination of pantothenic acid in an elemental diet by column-switching high-performance liquid chromatography with ultraviolet detection, *Anal.Sci.*, **1993**, *9*, 149–151.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate tablet in 40 mL mobile phase until it dissolves, make up to 50 mL with mobile phase, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES
Column: 200 mm long Nucleosil 7 C18
Mobile phase: Water:glacial acetic acid 95:5
Flow rate: 2
Injection volume: 100
Detector: RI

CHROMATOGRAM
Retention time: 4.50
Limit of detection: 500 ng/mL

KEY WORDS
tablets; detector temp 35

REFERENCE
Jonvel,P.; Andermann,G.; Barthelemy,J.F. Determination of calcium pantothenate in multivitamin preparations by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *281*, 371–376.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets if necessary. Add tablets containing 50 mg calcium pantothenate to 100 mL 5 mM pH 4.5 potassium phosphate buffer, sonicate at 75 W for 2 min, cool to room temperature, make up to 200 mL with buffer, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb NH2 aminopropyl
Mobile phase: MeCN:5 mM KH_2PO_4 87:13 (Wash column with MeCN:water 10:90 at the end of the day.)
Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: thiamine, riboflavin, niacinamide, pyridoxine

KEY WORDS
tablets

REFERENCE
Hudson,T.J.; Allen,R.J. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 113–115.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, add 80 mL water, shake at 240 oscillations/min for 30 min, make up to 100 mL with water, mix well, centrifuge at 1500 rpm, dilute supernatant with water to a concentration of 45 μg/mL, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 3:97 (Buffer was 250 mM NaH_2PO_4 adjusted to pH 2.5 with phosphoric acid.) (Flush column with MeCN:water 5:95 at the end of each day.)
Flow rate: 2
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 5
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: saccharin, pantoyllactone, degradation products
Interfering: panthenol

KEY WORDS
tablets; stability-indicating

REFERENCE
Timmons,J.A.; Meyer,J.C.; Steible,D.J.; Assenza,S.P. Reverse phase liquid chromatographic assay for calcium pantothenate in multivitamin preparations and raw materials, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 510–513.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS,, **1993**.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 0.9

OTHER SUBSTANCES
Simultaneous: niacin, pyridoxine, riboflavin, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 780.

SAMPLE
Matrix: solutions

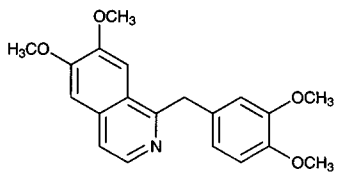
HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: biotin, folic acid, niacin, riboflavin, niacinamide

REFERENCE
MetaChem Catalog, **1995,** p. 21.

Papaverine



Molecular formula: $C_{20}H_{21}NO_4$
Molecular weight: 339.39
CAS Registry No.: 58-74-2, 61-25-6 (HCl)
Merck Index: 7151
Lednicer No.: 1 347

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 15 μL MeOH + 200 μL 4 M NaOH + 5 mL diethyl ether, vortex for 5 min, centrifuge at 2000 g for 10 min, remove the organic layer, extract the aqueous layer with 2 mL diethyl ether, centrifuge. Combine the organic layers and add them to 500 μL 1 M HCl, vortex for 1 min, centrifuge at 2000 g for 10 min. Remove the aqueous layer and add it to 500 μL 4 M NaOH, vortex for 1 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 25 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil 10 ODS
Mobile phase: MeOH:0.1% KH_2PO_4 65:35
Flow rate: 2
Detector: UV 238

CHROMATOGRAM
Retention time: 3.5
Internal standard: papaverine

OTHER SUBSTANCES
Extracted: ethaverine

KEY WORDS
plasma; papaverine is IS

REFERENCE
Brodie,R.R.; Chasseaud,L.F.; Walmsley,L.M.; Soegtrop,H.H.; Darragh,A.; O'Kelly,D.A. Determination of the antispasmodic agent ethaverine in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 379–386.

SAMPLE
Matrix: blood
Sample preparation: 4 mL Whole blood + 100 μL 8 μg/mL mepyramine maleate in water (prepare fresh daily), vortex, add 10 mL pH 10.0 phosphate buffer (μ = 0.4), vortex, add 5 mL chloroform:hexane 40:60, shake gently horizontally for 30 min, centrifuge. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Micropak CN-10
Mobile phase: n-Hexane:dichloromethane:MeCN:propylamine 50:25:25:0.1
Column temperature: 30
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 3.4
Internal standard: pyrilamine maleate (mepyramine maleate) (4.0)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: carbetapentane, cocaine, diamorphine, dioxyline, ethaverine, fluphenazine, imipramine, papaveraldine, promethazine, strychnine, thonzylamine
Interfering: methapyrilene, procaine, yohimbine

KEY WORDS
whole blood; pharmacokinetics

REFERENCE
Hoogewijs,G.; Michotte,Y.; Lambrecht,J.; Massart,D.L. High-performance liquid chromatographic determination of papaverine in whole blood, *J.Chromatogr.*, **1981**, *226*, 423–430.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 100 μg/mL noscapine + 1 mL 100 mM HCl + 6 mL dichloromethane, extract, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: Lichrosorb Si-60
Mobile phase: Hexane:dichloromethane:MeOH:diethylamine 95:4:1:0.03
Injection volume: 40
Detector: F ex 238 no emission filter

CHROMATOGRAM
Internal standard: noscapine
Limit of detection: 5 ng/mL

KEY WORDS
plasma; normal phase; pharmacokinetics

REFERENCE
Berg,G.; Jonsson,K.-Å.; Hammar,M.; Norlander,B. Variable bioavailability of papaverine, *Pharmacol.Toxicol.*, **1988**, *62*, 308–310.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane:isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN: water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: Gradient A was MeCN. B was 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min.
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 14
Limit of detection: 0.05 ppm

OTHER SUBSTANCES
Extracted: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, pentazocine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS
whole blood

REFERENCE
Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 300 μL 200 μg/mL laudanosine, mix, add 10 mL chloroform:isopropanol 95:5, vortex for 2 min, centrifuge for 30 min. Remove 8 mL of the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C8 (Brownlee)
Mobile phase: MeOH:15 mM pH 8.5 sodium borate buffer 58:42
Flow rate: 2.7
Injection volume: 20
Detector: UV 239

CHROMATOGRAM
Retention time: 5.0
Internal standard: laudanosine (9.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Noninterfering: caffeine, chlorothiazide, gentamicin, hydrochlorothiazide, oxytetracycline, tetracycline, theobromine, theophylline

KEY WORDS
plasma

REFERENCE
Gautam,S.R.; Nahum,A.; Baechler,J.; Bourne,D.W. Determination of papaverine in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 482–486.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a Toxiclean SPE cartridge (Alltech) with 3 mL MeOH, two 3 mL portions of water, and 2 mL buffer. 100 μL Plasma or serum + 100 μL MeOH + 200 μL MeCN + 100 μL buffer, vortex for 1 min, centrifuge at 4000 rpm for 15 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2.5 μg/mL flufenamic acid in MeOH (?), inject an aliquot. Urine. Condition a Bond Elut C8 SPE cartridge with 3 mL MeOH, two 3 mL portions of water, and 2 mL buffer. 100 μL Urine + 100 μL MeOH + 200 μL MeCN + 500 μL buffer, vortex for 1 min, centrifuge at 2000 rpm for 5 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2.5 μg/mL flufenamic acid in MeOH (?), inject an aliquot. (Buffer was 250 mL 25 mM sodium borate and 18 mL 100 mM NaOH, pH 9.2.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere HS C18
Mobile phase: MeCN:MeOH:1.2% ammonium acetate 15:40:45
Flow rate: 0.8
Detector: UV 239

CHROMATOGRAM
Retention time: 19.06
Internal standard: flufenamic acid (24.39)
Limit of quantitation: 60 ng/mL (urine), 20 ng/mL (plasma, serum)

OTHER SUBSTANCES
Extracted: codeine, monoacetylmorphine, morphine

KEY WORDS
SPE; plasma; serum

REFERENCE
Theodoridis,G.; Papadoyannis,I.; Tsoukali-Papadopoulou,H.; Vasilikiotis,G. A comparative study of different solid phase extraction procedures for the analysis of alkaloids of forensic interest in biological fluids by RP-HPLC/Diode array, *J.Liq.Chromatogr.*, **1995**, *18*, 1973–1975.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 251.1

CHROMATOGRAM
Retention time: 12.12

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 29.6

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, caffeine, cocaine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, procaine

REFERENCE
Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE
Matrix: cells
Sample preparation: 100 μL Cell suspension + 100 μL cefoperazone solution + 100 μL Hanks balanced salt solution, sonicate 30 min, add 800 μL MeCN, centrifuge at 13000 g for 5 min, remove supernatant. Dry supernatant under air, dissolve in 100 μL mobile phase, inject 75 μL.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:50 mM pH 4.7 $KH_2PO_4$40:60
Flow rate: 1
Injection volume: 75
Detector: UV 340

CHROMATOGRAM
Retention time: 9.5
Internal standard: rifampin
Limit of detection: 100-1000 ng/mL

REFERENCE
Darouiche,R.O.; Hamill,R.J. Antibiotic penetration of and bactericidal activity within endothelial cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1059–1064.

SAMPLE
Matrix: formulations
Sample preparation: Ampules. Dilute a 2 mL aliquot to 100 mL with buffer, dilute further with water to a papaverine concentration of 60 μg/mL, inject a 20 μL aliquot. Tablets. Crush a tablet, shake with 70 mL buffer for 10 min, make up to 100 mL with buffer, filter, dilute a 10 mL aliquot to 50 mL with water, inject a 20 μL aliquot. (The buffer was 1.248% NaH_2PO_4 adjusted to pH 3 with orthophosphoric acid.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: MeCN:buffer 70:30 (Buffer contained 2.88% sodium lauryl sulfate and 1.248% NaH_2PO_4 adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.16

OTHER SUBSTANCES
Simultaneous: moxaverine, drotaverine, ethaverine, codeine

KEY WORDS
ampules; tablets

REFERENCE
Girgis,E.H. Ion-pair reversed-phase liquid chromatographic identification and quantitation of papaverine congeners, *J.Pharm.Sci.*, **1993**, *82*, 503–505.

SAMPLE
Matrix: formulations
Sample preparation: Dilute syrup with mobile phase to a concentration of 5-100 μg/mL, shake, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 80 Å Ultrasphere CN
Mobile phase: MeCN:water:EtOH 60:38:2 containing 1 mM perchloric acid

Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: Conductivity, zero suppression 2, range 1 or 10

CHROMATOGRAM
Retention time: 12.1

OTHER SUBSTANCES
Simultaneous: bromhexine, chlorpheniramine, codeine, dextromethorphan, diphenhydramine, ephedrine, phenylephrine

KEY WORDS
syrup; indirect conductometric detection; presence of compound causes a decrease in mobile phase conductivity

REFERENCE
Lau,O.-W.; Mok,C.-S. High-performance liquid chromatographic determination of active ingredients in cough-cold syrups with indirect conductometric detection, *J.Chromatogr.A*, **1995**, *693*, 45–54.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.53

OTHER SUBSTANCES
Simultaneous: phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, naloxone, dextropropoxyphene, nalorphine, phenazocine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova Pak C18
Mobile phase: MeCN:50 mM pH 5.5 phosphate buffer 25:75
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 8.9

OTHER SUBSTANCES

Simultaneous: phentolamine

REFERENCE

Wang,D.-P.; Tu,Y.-H.; Allen,L.V.,Jr. Degradation kinetics of phentolamine hydrochloride in solution, *J.Pharm.Sci.*, **1988**, *77*, 972–976.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.16 μm PolyEncap ODS (n-octadecylacrylate copolymerized with vinyl silica in heptane, carrier Ultrasep ES 100; preparation described in paper)
Mobile phase: MeCN:pH 2.2 phosphate buffer 20:80
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: atropine, barbituric acid, codeine, diphenhydramine, noscapine

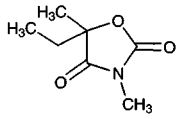
REFERENCE
Engelhardt,H.; Cuñat-Walter,M.A. Polymer encapsulated stationary phases with improved efficiency, *Chromatographia*, **1995**, *40*, 657–661.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge at 10000 rpm, dilute the supernatant with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil CPS
Mobile phase: MeCN:20 mM KH_2PO_4 50:50
Detector: UV 254

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

Paramethadione



Molecular formula: $C_7H_{11}NO_3$
Molecular weight: 157.17
CAS Registry No.: 115-67-3
Merck Index: 7161
Lednicer No.: 1 232

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 50 μL 7 μg/mL IS in water + 1 mL buffer, vortex for 10 s, add 5 mL n-hexane:ether:n-propanol 49:49:2, shake gently for 20 min, centrifuge at 1000 g for 5 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL mobile phase, inject a 50-100 μL aliquot. (Buffer was 10 mM sodium acetate:10 mM acetic acid 88.5:11.5, pH 5.5.) [Note: Extraction of paramethadione is implied but not explicitly stated.]

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeCN:buffer 28:72 (Buffer was 300 μL 1 M KH_2PO_4 and 50 μL 900 mM phosphoric acid in 1.8 L water, pH 4.4.)
Column temperature: 50
Flow rate: 2.8
Injection volume: 50-100
Detector: UV 195

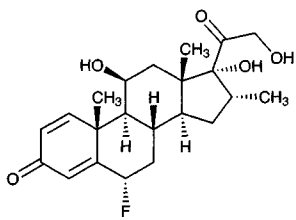
CHROMATOGRAM
Retention time: 3.8
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (11.5)

OTHER SUBSTANCES
Extracted: carbamazepine, ethosuximide, phenytoin, secobarbital
Simultaneous: mephobarbital, phenobarbital, primidone
Noninterfering: chlorazepate, clonazepam, diazepam, thioridazine, valproic acid

KEY WORDS
serum

REFERENCE
Levine,H.L.; Cohen,M.E.; Duffner,P.K.; Kustas,K.A.; Shen,D.D. An improved high-pressure liquid chromatographic assay for secobarbital in serum, *J.Pharm.Sci.*, **1982**, *71*, 1281–1283.

Paramethasone



Molecular formula: $C_{22}H_{29}FO_5$
Molecular weight: 392.47
CAS Registry No.: 53-33-8, 1597-82-6 (acetate)
Merck Index: 7162
Lednicer No.: 1 200

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM
Retention time: 19.19
Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)
Limit of detection: 1-10 ng/mL (245 nm)

OTHER SUBSTANCES
Extracted: betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluendrenolide, fluocinolone acetonide, fluorometholone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, prednisolone, prednisone, progesterone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone
Interfering: fluprednisolone

KEY WORDS
serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J.Chromatogr.B*, **1995**, *666*, 347–353.

Parathyroid hormone

Molecular weight: ca. 9500
CAS Registry No.: 902-64-6
Merck Index: 7168

SAMPLE
Matrix: reaction mixtures
Sample preparation: Prepare a 40 μM solution in 100 mM pH 10.0 borate/NaOH/HCl buffer, add hydrogen peroxide to a concentration of 1 mM, inject an aliquot.

HPLC VARIABLES
Column: 150 × 6.4 YMC-Pack ODS-A A312 (YMC)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN:water 60:40. A:B from 60:40 to 50:50 over 10 min, to 40:60 over 40 min.
Flow rate: 1
Detector: UV 215

CHROMATOGRAM
Retention time: 41

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
recombinant

REFERENCE
Nabuchi,Y.; Fujiwara,E.; Ueno,K.; Kuboniwa,H.; Asoh,Y.; Ushio,H. Oxidation of recombinant human parathyroid hormone: Effect of oxidized position on the biological activity, *Pharm.Res.*, **1995**, *12*, 2049–2052.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-Hypersil
Mobile phase: Gradient. A was 155 mM NaCl containing 10 mM HCl, pH 2.1. B was MeCN. A:B 100:0 for 2.5 min, to 90:10 over 2.5 min, to 40:60 over 67 min.
Column temperature: 45
Flow rate: 1
Detector: UV 215 or bioassay

CHROMATOGRAM
Retention time: 40.5 (human), 43 (cow)

REFERENCE
Zanelli,J.M.; Kent,J.C.; Rafferty,B.; Nissenson,R.A.; Nice,E.C.; Capp,M.W.; O'Hare,M.J. High-performance liquid chromatographic methods for the analysis of human parathyroid hormone in reference standards, parathyroid tissue and biological fluids, *J.Chromatogr.*, **1983**, *276*, 55–68.

SAMPLE
Matrix: solutions

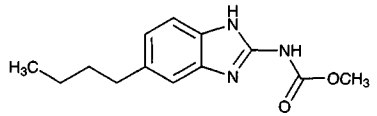
HPLC VARIABLES
Column: 250 × 4.6 RP C18 (Vydac)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was MeCN:0.1% aqueous trifluoroacetic acid 70:30. A: from 65:35 to 45:55 in 48 min, to 0:100 (step gradient), after 10 min return to initial conditions.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 32

KEY WORDS
human; recombinant

REFERENCE
Hogset,A.; Blingsmo,O.R.; Saether,O.; Gautvik,V.T.; Holmgren,E.; Hartmanis,M.; Josephson,S.; Gabrielsen,O.S.; Gordeladze,J.O.; Alestrom,P.; Gautvik,K.M. Expression and characterization of a recombinant human parathyroid hormone secreted by Escherichia coli employing the staphylococcal protein A promoter and signal sequence, *J.Biol.Chem.*, **1990**, *265*, 7338–7344.

Parbendazole



Molecular formula: $C_{13}H_{17}N_3O_2$
Molecular weight: 247.30
CAS Registry No.: 14255-87-9
Merck Index: 7169

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

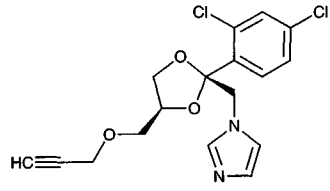
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM
Retention time: 18.15

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Parconazole



Molecular formula: $C_{17}H_{16}Cl_2N_2O_3$
Molecular weight: 367.23
CAS Registry No.: 61400-59-7, 62973-77-7 (HCl)
Lednicer No.: 3 133

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

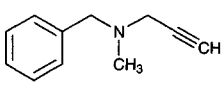
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 15.5

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pargyline



Molecular formula: $C_{11}H_{13}N$
Molecular weight: 159.23
CAS Registry No.: 555-57-7, 306-07-0 (HCl)
Merck Index: 7172

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

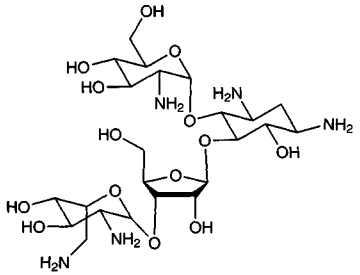
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Paromomycin



Molecular formula: $C_{23}H_{45}N_5O_{14}$
Molecular weight: 615.64
CAS Registry No.: 7542-37-2, 1263-89-4 (sulfate)
Merck Index: 7173

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 300 μL Plasma + 30 μL 101.4 μg/mL kanamycin B in water + 100 μL 2 M perchloric acid, vortex for 2-3 s, centrifuge at 1000 g for 5 min. Remove the supernatant and neutralize it with 1.5 M NaOH, add 300 μL buffer, add 400 μL DMSO, add 100 μL 2% 2,4-dinitrofluorobenzene in EtOH, vortex, heat at 64° for 30 min, add 3 mL toluene, vortex, centrifuge, discard the upper toluene layer, add 3 mL MeCN:toluene 50:50, vortex for 5-10 s. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 1 mL MeCN:water 50:50, inject a 20 μL aliquot. Urine. Dilute urine 100-fold with water. 300 μL Diluted urine + 30 μL 101.4 μg/mL kanamycin B in water + 300 μL buffer + 400 μL DMSO + 100 μL 2% 2,4-dinitrofluorobenzene in EtOH, vortex, heat at 64° for 30 min, add 3 mL toluene, vortex, centrifuge, discard the upper toluene layer, add 3 mL MeCN:toluene 50:50, vortex for 5-10 s. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 1 mL MeCN: water 50:50, inject a 20 μL aliquot. (Prepare buffer by mixing 80 mL 100 mM Na_2HPO_4 and 20 mL 100 mM NaH_2PO_4, adding 1 g Tris HCl, and adjusting the pH to 7.8 with 6 M HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax SB-C18
Mobile phase: MeOH:water 64:36, adjusted to pH 3.0 with phosphoric acid
Column temperature: 50
Flow rate: 2
Injection volume: 10-20
Detector: UV 350

CHROMATOGRAM
Retention time: 14.0
Internal standard: kanamycin B (24.0)
Limit of detection: 200 ng/mL (plasma), 500 ng/mL (urine)
Limit of quantitation: 500 ng/mL (plasma), 1 μg/mL (urine)

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Lu,J.; Cwik,M.; Kanyok,T. Determination of paromomycin in human plasma and urine by reversed-phase high-performance liquid chromatography using 2,4-dinitrofluorobenzene derivatization, *J.Chromatogr.B*, **1997**, *695*, 329–335.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 2 mg/mL solution in 20 mM pH 9.0 borate buffer, remove a 5 mL aliquot and add it to 15 mL 150 mM 2,4-dinitrofluorobenzene in MeOH (prepare fresh

daily), heat at 100° for 45 min, cool, make up to 250 mL with mobile phase, discard the upper aqueous phase, inject a 20 μL aliquot of the lower organic phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb SI-100
Mobile phase: Chloroform:THF:water 25:28.2:0.8
Flow rate: 1
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 12,25

KEY WORDS
normal phase; derivatization

REFERENCE
Tsuji,K.; Goetz,J.F.; VanMeter,W.; Gusciora,K.A. Normal-phase high-performance liquid chromatographic determination of neomycin sulfate derivatized with 1-fluoro-2,4-dinitrobenzene, *J.Chromatogr.*, **1979**, *175*, 141–152.

SAMPLE
Matrix: formulations
Sample preparation: Mix 2 g cream with 3 mL n-butanol, add 5 mL 2% sulfuric acid, mix thoroughly. Separate lower aqueous layer and re-extract the organic layer with another portion of sulfuric acid. Combine the aqueous layers and make up to 100 mL with water. Filter a portion of the extract through a 0.45 μm Nylon 66 syringe filter. Dilute filtrate with water, inject a 10 μL. aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Metachem Inertsil C8
Mobile phase: 200 mM Sodium sulfate containing 1.2 mM sodium 1-heptanesulfonate and 0.1% acetic acid
Flow rate: 1
Injection volume: 10
Detector: F ex 340 em 440 following post-column reaction. The column effluent mixed with reagent pumped at 1.0 mL/min and this mixture flowed through a 9 m × 0.25 mm I.D. stainless steel coil to the detector. (Reagent was 800 mg o-phthalaldehyde and 1 mL mercaptoethanol in 10 mL MeOH diluted to 1 L with 2.5% boric acid and adjusted to pH 10 with 2.5% KOH.)

CHROMATOGRAM
Retention time: 21.5
Limit of detection: 8 ng

OTHER SUBSTANCES
Also analyzed: gentamicin

KEY WORDS
cream; post-column reaction

REFERENCE
Pick,J.; Olson,L.L.; Ellis,W.Y.; Lim,P. Development and validation of a method to extract and quantitate paromomycin and gentamicin from an Aquaphilic cream formulation, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 131–137.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:water 17:83 containing 16 mM sodium 1-hexanesulfonate 20 mM Na_3PO_4, pH 3.5 (Connect a 250 × 4.6 column of Bondapak C18/Corasil or Co:Pell ODS between pump and injector. Flush column with MeOH:water 50:50 at the end of the day.)
Column temperature: 25
Flow rate: 1.5
Injection volume: 25
Detector: RI

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: neomycin

REFERENCE
Whall,T.J. Determination of streptomycin sulfate and dihydrostreptomycin sulfate by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *219*, 89–100.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 8 μm PLRP-S 1000 Å poly(styrene-divinylbenzene) (Polymer Laboratories)
Mobile phase: Water containing 70 g/L sodium sulfate, 1.4 g/L sodium 1-octanesulfonate, and 50 mL/L 200 mM pH 3.0 phosphate buffer
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: E, Dionex PED-1 pulsed electrochemical detector at 35°, 3 mm dia. gold working electrode, E_1 +0.05 V, E_2 +0.75 V, E_3 -0.15 V, t_1 0-0.40 s, t_2 0.41-0.60 s, t_3 0.61-1.00 s, measure signal between 0.2 and 0.4 s, stainless steel counter electrode, Ag/AgCl reference electrode, following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.3 mL/min and the mixture flowed through a 1.2 m long 500 μL coil to the detector. (Prepare 500 mM NaOH solution by diluting 50% NaOH with helium-degassed water. Clean gold electrode after each 60 analyses.)

CHROMATOGRAM
Retention time: 9 (paromomycin II), 11 (paromomycin I)
Limit of detection: 5 ng
Limit of quantitation: 15 ng

OTHER SUBSTANCES
Simultaneous: neamine, neomycin B, neomycin C, neomycin LP-A, neomycin LP-B, paromamine

KEY WORDS
post-column reaction

REFERENCE
Adams,E.; Schepers,R.; Roets,E.; Hoogmartens,J. Determination of neomycin sulfate by liquid chromatography with pulsed electrochemical detection, *J.Chromatogr.A*, **1996**, *741*, 233–240.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Tissumizer) 1 g Ground tissue + 4 mL buffer at medium speed for 1 min, centrifuge at 3600 g for 20 min, remove the supernatant, re-homogenize pellet in 4 mL buffer for 10 min, centrifuge. Combine the supernatants, heat in a boiling water bath with occasional mixing for 5 min, centrifuge at 2000 g for 20 min, remove the supernatant, vortex the precipitate with 2 mL buffer for 30 s, centrifuge at 2000 g for 10 min. Combine the supernatants, acidify to pH 3.5-4 with 50-60 μL sulfuric acid, centrifuge at 2000 g for 10 min,

inject an aliquot of the supernatant. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water, pH 8.0.)

HPLC VARIABLES
Guard column: 10 μm RP-18
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 1.5:98.5 (Buffer was 10 mM sodium 1-pentanesulfonate, 56 mM sodium sulfate, and 7 mM acetic acid.)
Flow rate: 1.5
Detector: F ex 340 em 455 following post-column reaction with derivatization reagent pumped at 0.9 mL/min. (Derivatization reagent was commercially available (Pierce) or prepared by adding 2.5 mL 2-mercaptoethanol and 2.5 mL Brij-35 to 850 mg o-phthalaldehyde in 10 mL MeOH, mix until decolorization is complete, add 1 L buffer, filter (0.45 μm), and refrigerate until used. Buffer was prepared by adjusting pH of 250 mM boric acid to 9.5 with 5 M KOH.)

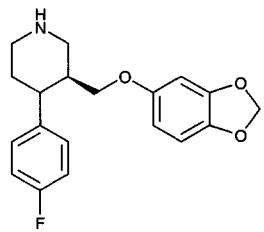
CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Extracted: neomycin
Simultaneous: dihydrostreptomycin, streptomycin

KEY WORDS
kidney; muscle; cow; pig; post-column reaction

REFERENCE
Shaikh,B.; Allen,E.H.; Gridley,J.C. Determination of neomycin in animal tissues, using ion-pair liquid chromatography with fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 29–36.

Paroxetine



Molecular formula: $C_{19}H_{20}FNO_3$
Molecular weight: 329.37
CAS Registry No.: 61869-08-7
Merck Index: 7175
Lednicer No.: 5 87

SAMPLE
Matrix: blood
Sample preparation: Condition a 50 mg Carboxymethyl Isolute SPE cartridge with 1 mL MeOH and 1 mL 25 mM pH 6.8 phosphate buffer, dry under vacuum. Add 500 μL plasma to the SPE cartridge, wash with two 1 mL portions of 25 mM pH 6.8 phosphate buffer, dry under vacuum, elute with 1 mL 1% ammonia in MeOH, evaporate to dryness under vacuum at 40°, reconstitute the residue in 100 μL MeOH, vortex, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS/CN
Mobile phase: MeOH:50 mM pH 4.8 potassium phosphate buffer 70:30
Flow rate: 1
Injection volume: 25
Detector: E, ESA, Model 5100 A, Model 5010 analytical cell +650 mV on channel 1, +950 mV on channel 2, Model 5020 guard cell +980 mV

CHROMATOGRAM
Retention time: 9.6
Internal standard: paroxetine